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OCT 24 2002

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SEQUENCE LISTING

<110> Meares, Claude
Chmura, Albert
The Regents of the University of California

<120> Engineering Antibodies That Bind Irreversibly

<130> 023070-099120US

<140> US 09/671,953

<141> 2000-09-27

<150> US 60/156,194

<151> 1999-09-27

<150> US 60/208,684

<151> 2000-05-31

<160> 23

<170> PatentIn Ver. 2.1

<210> 1

<211> 753

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes Fab heavy chain of CHA255

<400> 1

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aaactctcct gtgcagcctc tggattcact ttaagtgggtg aaaccatgtc ttgggttcgc 120
cagactccgg agaagagggt ggagtgggtc acaaccactc ttagtgggtg tggtttcacc 180
ttctattcag ccagtgtgaa gggtcgtttc accatctcca gagacaatgc ccagaacaac 240
ctctatctac aactgaatag tctgaggtct gaggacacgg ccttgatatt ctgtgcaagt 300
catcggtttg ttcactgggg ccacgggact ctggtcactg tctctgcagc caaaacgacg 360
ggcccacggg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc 420
ctgggctgcc tggcaagga ctacttcccc gaaccgggtga cgggtgctgt gaactcaggc 480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcaag actctacttc 540
ctcagcagcg tggtgaccgt gcccttcaac agcttgggca cccagaccta catctgcaac 600
gtgaatcaca agcccagcaa caccaagggtg gacaagaaag cagagcccaa atcttgtagac 660
aaatctagag ggcccttcga aggtaagcct atccctaacc ctctcctcgg tctcgattct 720
acgcgtaccg gtcacatca ccatcaccat tga 753

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<210> 2

<211> 657

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes light chain mutant with Cys
substituted for Asn at position 97 of CHA255

<400> 2

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agatctgctg ttgtgactca ggaatctgca ctcaccacat cacctgggtga aacagtcaca 60
ctcacttgct gctcaagtat tggggctggt acaactagta actatgccaa ctgggtccaa 120

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gaaaaaccag atcattttatt cactgggtcta ataggtggta ccaataaccg ggctccgggt 180
gttcttgcca gattctcagg ctccctgatt ggagacaagg ctgccctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tgtgctctat ggtactcctg cctctgggtr 300
ttcgggtggag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccg cactctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtggagg tggataacgc cctccaatcg 480
ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600
acccatcagg gcctgagyty gcccgtcaca aagagcttca acaggggaga gtgttaa 657

<210> 3

<211> 657

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes the unmodified light chain of CHA255

<400> 3

agatctgctg ttgtgactca ggaatctgca ctcaccacat cacctgggtga aacagtcaca 60
ctcacttgctc gctcaagtat tggggctggt acaactagta actatgccaa ctgggtccaa 120
gaaaaaccag atcattttatt cactgggtcta ataggtggta ccaataaccg ggctccgggt 180
gttcttgcca gattctcagg ctccctgatt ggagacaagg ctgccctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tgtgctctat ggtactccaa cctctgggtr 300
ttcgggtggag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccg cactctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtggagg tggataacgc cctccaatcg 480
ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600
acccatcagg gcctgagyty gcccgtcaca aagagcttca acaggggaga gtgttaa 657

<210> 4

<211> 657

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:nucleic acid
that encodes light chain mutant with Cys
substituted for Ser at position 96 of CHA255

<400> 4

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ctcacttgctc gctcaagtat tggggctggt acaactagta actatgccaa ctgggtccaa 120
gaaaaaccag atcattttatt cactgggtcta ataggtggta ccaataaccg ggctccgggt 180
gttcttgcca gattctcagg ctccctgatt ggagacaagg ctgccctcac catcacaggg 240
gcacagactg aagatgaggc aagatatttc tgtgctctat ggtactgcaa cctctgggtr 300
ttcgggtggag gaaccaaact gactgtccta agccgwackg tggctgcacc atctgtcttc 360
atcttcccg cactctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420
aataacttct atcccagaga ggccaaagta cagtggagg tggataacgc cctccaatcg 480
ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540
agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600
acccatcagg gcctgagyty gcccgtcaca aagagcttca acaggggaga gtgttaa 657

<210> 5
 <211> 218
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: polypeptide
 sequence of mutant light chain with Cys
 substituted for Asn at position 97 of CHA255

<220>
 <221> MOD_RES
 <222> (207)
 <223> Xaa = any amino acid

<400> 5
 Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
 1 5 10 15
 Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
 20 25 30
 Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
 35 40 45
 Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
 50 55 60
 Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
 65 70 75 80
 Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Ser
 85 90 95
 Cys Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 6
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide
sequence of unmodified light chain of CHA255

<220>
<221> MOD_RES
<222> (207)
<223> Xaa = any amino acid

<400> 6
Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
1 5 10 15
Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
20 25 30
Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
35 40 45
Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
50 55 60
Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
65 70 75 80
Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Ser
85 90 95
Asn Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 7
 <211> 218
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: polypeptide
 sequence of mutant light chain with Cys
 substituted for Ser at position 96 of CHA255

<220>
 <221> MOD_RES
 <222> (207)
 <223> Xaa = any amino acid

<400> 7
 Arg Ser Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly
 1 5 10 15
 Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr
 20 25 30
 Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr
 35 40 45
 Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg
 50 55 60
 Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly
 65 70 75 80
 Ala Gln Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys
 85 90 95
 Asn Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Xaa Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 8
<211> 250
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: polypeptide
sequence of unmodified heavy chain of CHA255

<400> 8

Arg Ser Glu Val Thr Leu Val Glu Ser Arg Gly Asp Ser Val Lys Pro
1 5 10 15
Gly Gly Phe Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser
20 25 30
Gly Glu Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu
35 40 45
Trp Val Thr Thr Thr Leu Ser Gly Gly Gly Phe Thr Phe Tyr Ser Ala
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn
65 70 75 80
Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr
85 90 95
Phe Cys Ala Ser His Arg Phe Val His Trp Gly His Gly Thr Leu Val
100 105 110
Thr Val Ser Ala Ala Lys Thr Thr Gly Pro Ser Val Phe Pro Leu Ala
115 120 125
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130 135 140
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175
Arg Leu Tyr Phe Leu Ser Ser Val Val Thr Val Pro Phe Asn Ser Leu
180 185 190
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205
Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Ser Arg Gly
210 215 220
Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser
225 230 235 240
Thr Arg Thr Gly His His His His His His
245 250

<210> 9
 <211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:T7 promoter
 primer

 <400> 9
 ctaatacgac tcactatagg g 21

 <210> 10
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:K XbaI primer

 <400> 10
 ctgcaggctcg actctagagg atctactagt 30

 <210> 11
 <211> 35
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:mutagenic site

 <400> 11
 catgcctgca ggtcgactct agaggatcta ctagt 35

 <210> 12
 <211> 39
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:mutagenic site

 <400> 12
 ttctgtgctc tatggtacag caacctctgg gtattcggt 39

 <210> 13
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:mutagenesis
 primer S95C

 <400> 13
 ataccagag gttgcagtac catagagcac 30

<210> 14
 <211> 19
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:U-19 primer

 <400> 14
 gggttttccca gtcacgacg 19

 <210> 15
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:mutagenesis
 primer N96C

 <400> 15
 ataccacagag gcagctgtac catagagcac 30

 <210> 16
 <211> 360
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:V-H sequence of
 CHA255

 <220>
 <221> CDS
 <222> (1)..(360)

 <400> 16
 gaa gtg acg ctg gtg gag tct ggg gga gac tca gtg aag cct gga ggg 48
 Glu Val Thr Leu Val Glu Ser Gly Gly Asp Ser Val Lys Pro Gly Gly
 1 5 10 15

 tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act tta agt ggt gaa 96
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Gly Glu
 20 25 30

 acc atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc 144
 Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45

 gca acc act ctt agt ggt ggt ggt ttc acc ttc tat tca gcc agt gtg 192
 Ala Thr Thr Leu Ser Gly Gly Gly Phe Thr Phe Tyr Ser Ala Ser Val
 50 55 60

 aag ggt cgt ttc acc atc tcc aga gac aat gcc cag aac aac ctc tat 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn Leu Tyr
 65 70 75 80

cta	caa	ctg	aat	agt	ctg	agg	tct	gag	gac	acg	gcc	ttg	tat	ttc	tgt	288
Leu	Gln	Leu	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Phe	Cys	
			85						90					95		

gca	agt	cat	cgg	ttt	gtt	cac	tgg	ggc	cac	ggg	act	ctg	gtc	act	gtc	336
Ala	Ser	His	Arg	Phe	Val	His	Trp	Gly	His	Gly	Thr	Leu	Val	Thr	Val	
			100					105					110			

tct	gca	gcc	aaa	acg	aca	ccc	cca									360
Ser	Ala	Ala	Lys	Thr	Thr	Pro	Pro									
			115				120									

<210> 17
 <211> 120
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence:V-H sequence of
 CHA255

<400> 17																
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1				5					10					15		
Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Leu	Ser	Gly	Glu	
			20					25					30			
Thr	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	
			35				40					45				
Ala	Thr	Thr	Leu	Ser	Gly	Gly	Gly	Phe	Thr	Phe	Tyr	Ser	Ala	Ser	Val	
			50			55					60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Gln	Asn	Asn	Leu	Tyr	
	65				70				75					80		
Leu	Gln	Leu	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Phe	Cys	
			85						90					95		
Ala	Ser	His	Arg	Phe	Val	His	Trp	Gly	His	Gly	Thr	Leu	Val	Thr	Val	
			100					105					110			
Ser	Ala	Ala	Lys	Thr	Thr	Pro	Pro									
			115				120									

<210> 18
 <211> 28
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence:cloning primer
 with XhoI site

<400> 18																
ggtgctcgag	tctgggggag	actcagtg														28

<210> 19
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:cloning primer
 with ApaI site

<400> 19
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26

<210> 20
 <211> 405
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:V-L sequence of
 CHA255 mutant S95C

<220>
 <221> CDS
 <222> (1)..(405)

<220>
 <221> modified_base
 <222> (405)
 <223> n = g, a, c or t

<400> 20
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 1 5 10 15
 gtc aca ctc act tgt cgc tca agt att ggg gct gtt aca act agt aac 96
 Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn
 20 25 30
 tat gcc aac tgg gtc caa gaa aaa cca gat cat tta ttc act ggt cta 144
 Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu
 35 40 45
 ata ggt ggt acc aat aac cgg gct ccg ggt gtt cct gcc aga ttc tca 192
 Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser
 50 55 60
 ggc tcc ctg att gga gac aag gct gcc ctc acc atc aca ggg gca cag 240
 Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
 65 70 75 80
 act gaa gat gag gca aga tat ttc tgt gct cta tgg tac tgc aac ctc 288
 Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu
 85 90 95
 tgg gtg ttc ggt gga gga acc aaa ctg act gtc cta agc cag ccc aag 336
 Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys
 100 105 110

tct tcg cca tca gtc acc ctg ttt ccg ccc tcc tct gaa gag cta agc 384
 Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Ser
 115 120 125

ttg gga atc gga ttc ccg ggn 405
 Leu Gly Ile Gly Phe Pro Gly
 130 135

<210> 21
 <211> 135
 <212> PRT
 <213> Artificial Sequence
 <223> Description of Artificial Sequence:V-L sequence of
 CHA255 mutant S95C

<400> 21
 Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr
 1 5 10 15
 Val Thr Leu Thr Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn
 20 25 30
 Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu
 35 40 45
 Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser
 50 55 60
 Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
 65 70 75 80
 Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu
 85 90 95
 Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys
 100 105 110
 Ser Ser Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Ser
 115 120 125
 Leu Gly Ile Gly Phe Pro Gly
 130 135

<210> 22
 <211> 31
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence:cloning primer
 with SstI site

<400> 22
 ctcagagctc gctgttgtga ctcaggaatc t 31

<210> 23
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:cloning primer
with BsiWI site

<400> 23
ctcgcatgcg cttaggacag tcagttt

27